Amendments to the Specification

Please replace the title at page 1 of the specification with the following amended title:

B7-H1 ~~AND B7-DC~~ VARIANTS

Please replace the paragraph beginning at page 12, line 19 with the following amended paragraph:

In eukaryotic host cells, a number of viral-based expression systems can be utilized to express costimulatory polypeptides (e.g., variant B7-H1 and variant B7-DC polypeptides). A nucleic acid encoding a costimulatory polypeptide of the invention can be cloned into, for example, a baculoviral vector such as ~~pBlueBac~~ pBLUEBAC™ (Invitrogen Life Technologies, Carlsbad, CA), which can be used to co-transfect insect cells such as *Spodoptera frugiperda* (Sf9) cells with wild type DNA from *Autographa californica* multiply enveloped nuclear polyhedrosis virus (AcMNPV). Recombinant viruses producing costimulatory polypeptides can be identified by standard methodology. Alternatively, a nucleic acid encoding a costimulatory polypeptide such as variant B7-H1 or variant B7-DC can be introduced into an SV40, retroviral, or vaccinia based viral vector for infection of suitable host cells.

Please replace the paragraph beginning at page 12, line 29 with the following amended paragraph:

Mammalian cell lines that stably express variant costimulatory polypeptides can be produced using expression vectors with appropriate control elements and a selectable marker. For example, the eukaryotic expression vectors ~~pCR3.1~~ pCR®3.1 (Invitrogen Life Technologies) and p91023(B) (see Wong *et al.* (1985) *Science* 228:810-815) are suitable for expression of variant costimulatory polypeptides in, for example, Chinese hamster ovary (CHO) cells, COS-1 cells, human embryonic kidney 293 cells, NIH3T3 cells, BHK21 cells, MDCK cells, and human vascular endothelial cells (HUVEC). Following introduction of an expression vector by electroporation, lipofection, calcium phosphate, or calcium chloride co-precipitation, DEAE dextran, or other suitable transfection method, stable cell lines can be selected (e.g., by antibiotic resistance to G418, kanamycin, or hygromycin). The transfected cells can be cultured such that the polypeptide of interest is expressed, and the polypeptide can be recovered from, for example, the cell culture supernatant or from lysed cells. Alternatively, a variant costimulatory polypeptide can be produced by (a) ligating amplified sequences into a mammalian expression vector such as ~~pcDNA3~~ pcDNA™3 (Invitrogen Life Technologies), and (b) transcribing and translating *in vitro* using wheat germ extract or rabbit reticulocyte lysate.

Please replace the paragraph beginning at page 13, line 13 with the following amended paragraph:

Variant costimulatory polypeptides can be purified using, for example, chromatographic methods such as DEAE ion exchange, gel filtration, and hydroxylapatite chromatography. For example, a costimulatory polypeptide in a cell culture supernatant or a cytoplasmic extract can be purified using a protein G column. In some embodiments, variant costimulatory polypeptides can be "engineered" to contain an amino acid sequence that allows the polypeptides to be captured onto an affinity matrix. For example, a tag such as c-myc, hemagglutinin, polyhistidine, or ~~Flag™~~ FLAG™ (Kodak) can be used to aid polypeptide purification. Such tags can be inserted anywhere within the polypeptide, including at either the carboxyl or amino terminus. Other fusions that can be useful include enzymes that aid in the detection of the polypeptide, such as alkaline phosphatase. Immunoaffinity chromatography also can be used to purify costimulatory polypeptides.

Please replace the paragraph beginning at page 17, line 5 with the following amended paragraph:

An expression vector can include a tag sequence designed to facilitate subsequent manipulation of the expressed nucleic acid sequence (e.g., purification or localization). Tag sequences, such as green fluorescent protein (GFP), glutathione S-transferase (GST), polyhistidine, c-myc, hemagglutinin, or ~~Flag™~~ FLAG™ tag (Kodak, New Haven, CT) sequences typically are expressed as a fusion with the encoded polypeptide. Such tags can be inserted anywhere within the polypeptide including at either the carboxyl or amino terminus.

Please replace the paragraph beginning at page 21, line 30 with the following amended paragraph:

Another way to achieve uptake of a nucleic acid is to use liposomes, which can be prepared by standard methods known in the art, for example. Nucleic acid vectors can be incorporated alone into these delivery vehicles or can be co-incorporated with tissue-specific antibodies. Alternatively, a molecular conjugate can be prepared to contain a plasmid or other vector attached to poly-L-lysine by electrostatic or covalent forces. Poly-L-lysine binds to a ligand that can bind to a receptor on target cells (Cristiano *et al.* (1995) *J. Mol. Med.* 73:479-486). Alternatively, lymphoid tissue specific targeting can be achieved using lymphoid tissue-specific transcriptional regulatory elements (TREs) such as a B lymphocyte-, T lymphocyte-, or dendritic cell-specific TRE. Lymphoid tissue specific TREs include, for example, those known in the art [see, e.g., Thompson *et al.* (1992) *Mol. Cell. Biol.* 12:1043-1053; Todd *et al.* (1993) *J. Exp. Med.* 177:1663-1674; and Penix *et al.* (1993) *J. Exp. Med.* 178:1483-1496]. Delivery of [[:]] "naked DNA" (i.e., without a delivery vehicle) to an intramuscular, intradermal, or subcutaneous site is another means to achieve *in vivo* expression.

Please replace the paragraph beginning at page 25, line 23 with the following amended paragraph:

Molecular modeling: Molecular models of the Ig V-type domains of human B7-H1 (hB7-H1), mouse B7-H1 (mB7-H1), human B7-DC (hB7-DC), and mouse B7-DC (mB7-DC) were generated by homology (or comparative) modeling based on X-ray coordinates of human CD80 and CD86, as seen in the structures of the CD80/CTLA-4 and CD86/CTLA-4 complexes. First, the V-domains of CD80 and CD86 were optimally superimposed, and sequences of B7 family members were aligned based on this superimposition. The superimposition and initial alignments were carried out using the sequence-structure alignment function of MOE (Molecular Operating Environment, Chemical Computing Group, Montreal, Quebec, Canada). The alignment was then manually adjusted to match Ig consensus positions and to map other conserved hydrophobic residues in the target sequences to core positions in the X-ray structures.

Corresponding residues in the aligned sequences thus were predicted to have roughly equivalent spatial positions. Taking this kind of structural information into account typically is a more reliable alignment criterion than sequence identity alone if the identity is low, as in this case. In the aligned region, the average identity of the compared B7 sequences relative to the two structural templates, CD80 and CD86, was only approximately 16%. The final version of the structure-oriented sequence alignment, which provided the basis for model building, is shown in Figure 9. Following the alignment, core regions of the four models were automatically assembled with MOE from the structural templates, and insertions and deletions in loop regions were modeled by applying a segment matching procedure (Levitt (1992) *J. Mol. Biol.* 226:507-533; and Fechteler *et al.* (1995) *J. Mol. Biol.* 253:114-131). Side chain replacements were carried out using preferred rotamer conformations seen in high-resolution protein databank structures (Ponder and Richards (1987) *J. Mol. Biol.* 193:775-791; and Berman *et al.* (2000) *Nucl. Acids Res.* 28:235-242). In each case, twenty intermediate models were generated, average coordinates were calculated, and the resulting structures were energy minimized using a protein force field (Engh and Huber (1991) *Acta Cryst.* A47:392-400) until intramolecular contacts and stereochemistry of each model were reasonable. Graphical analysis of the models, including calculation of solvent-accessible surfaces (Connolly (1983) *J. Appl. Cryst.* 16:548-558) and residue mapping studies were carried out with ~~InsightII~~ INSIGHTII® (Accelrys, San Diego, California).

Please replace the paragraph beginning at page 27, line 30 with the following amended paragraph:

Surface Plasmon Resonance Analysis: The affinity of purified wild type and mutant B7-H1 and B7-DC polypeptides was analyzed on a ~~BIAcore™~~ BIACORE™ 3000 instrument (Biacore AB, Uppsala, Sweden). All reagents except fusion proteins were purchased, pre-filtered, and degassed from BIAcore. All experiments were performed at 25°C using 0.1 M HEPES, 0.15 M NaCl (pH 7.4) as a running buffer. Briefly, PD-1Ig was first immobilized onto a CM5 sensor chip (BIAcore) by amine coupling according to the BIAcore protocol. A flow cell of the CM5 chip was derivatized through injection of a 1:1 EDC:NHS [*N*-ethyl-*N'*-

(diethylaminopropyl) carbodiimide:*N*-hydroxysuccinimide] mixture for seven minutes, followed by injection of 20 µg/ml of PD-1Ig at 10 µl/min diluted in 10 mM sodium acetate (pH 4.5). The PD-1Ig was immobilized at 2000 RUs. This was followed by blocking the remaining activated carboxyl groups with 1 M ethanolamine (pH 8.5). A control flow cell was prepared in a similar fashion as above, substituting running buffer alone in place of PD-1Ig. The fusion proteins were diluted in running buffer in a concentration series of 3.75, 7.5, 15, 30, and 60 µg/ml. The proteins were injected at a flow rate of 20 µl/min for 3 minutes, and buffer was allowed to flow over the surface for 5 minutes for dissociation data. The flow cells were regenerated with a single 30-second pulse of 10 mM NaOH. Data analysis was performed using BIAevaluation software package 3.1 (BIAcore).

Please replace the paragraph beginning at page 28, line 16 with the following amended paragraph:

T cell proliferation and cytokine assays: T cells from wild type B6 mice or PD-$1^{-/-}$ mice were purified using nylon wool columns (Robbins Scientific Co, Sunnyvale, CA) as described previously (Wang *et al. supra*). The enriched T cells were cultured at 3 x 10^5 cells per well in flat-bottomed 96-well microplates that were pre-coated with anti-CD3 mAb (clone 145-2C11, Pharmingen, San Diego, CA) in the presence of 5 µg/ml of fusion or control polypeptides. Proliferation of T cells was determined by incorporation of 1 µCi/well ^{3}H-TdR during the last 12 hours of the 3-day culture. ^{3}H-TdR incorporation was counted using a ~~MicroBeta~~ MICROBETA® Trilux liquid scintillation counter (Wallac, Finland). To detect cytokine, culture supernatants were collected at various time points, and the concentration of IFN-γ was measured by sandwich ELISA following the manufacturer's instructions (Pharmingen).

Please replace the paper copy of the Sequence Listing submitted on March 18, 2004, with the paper copy submitted herewith.